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COMMUNICATION

Enantioenrichment in sublimed amino acid mixtures†‡

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A real amplification of an initial enantiomeric excess can be detected when two amino acids are sublimed at high temperature, even if one of the components is a racemic compound that does not convert into a conglomerate by sublimation.

It has now been recognized that physical processes such as crystallization, evaporation, or sublimation may lead to enantioenriched, even almost enantiopure, substances on the basis of thermodynamic and kinetic considerations.[1–4] These phase transitions possess indeed a prebiotic connotation and overcome the puzzle of chirogenesis resulting from prebiotic chemical reactions, which give a huge distribution of products in low yields as racemic mixtures. Such facile, physically-driven deracemizations may help us to explain why homochiral sequences, rather than heterochiral or racemic, were presumably present before the appearance of biochemical machineries,[5,6] and how RNA templates and protocells might have ultimately acquired a unichiral bias.§

Since the terrestrial biosphere is dominated by the presence of aqueous environments, crystallization, rather than sublimation, represents a major influence. However, sublimation is a process that would have been much more likely to occur in the cosmochemical regimes that saw the prebiotic formation of organic molecules.[7,8] Moreover, sublimation plays a key role in frozen planets with low or no atmospheric pressure leading to morphological textures that can be distinguished from those caused by melting.[9]

Differences in the vapour pressure (or heats of sublimation) between a racemic compound and its enantiomer constituents account for the enantioenrichment of several compounds after sublimation, as evidenced by experiments reported some decades ago[10] and revisited recently.[11] A series of clever variations have been introduced since, especially in the field of organofluorine compounds, such as the use of a sublimation enabling tag that facilitates enantiomer purification of non-volatile substances.[12] Guillemin and co-workers have also shown that partial sublimation of L-asparagine with other amino acids breaks the racemic state of the latter leading to small ees of L-enantioenriched residues (the sublimate is D-enriched).[13] These results are reminiscent of a previous strategy employed by Kojo and coworkers because asparagine (an amino acid that crystallizes as conglomerate) induces enantioselective crystallizations of other amino acids.[14] And such experiments constitute actually an extension of a more general process of surface recognition leading to mixed crystals with reduced symmetry.[15]

A different and more intriguing fact that also enables the assessment of the whole solid subjected to sublimation was found during the high-temperature sublimation of valine (that crystallizes as racemic compound) as this proteinogenic amino acid is converted into a conglomerate under such conditions. Since for resolutions of enantiomer mixtures to be possible, the racemate must be a conglomerate, valine samples enriched in either L- or D-enantiomers afforded a significant amplification of the ees when sublimed.[16] Although such a racemic compound–conglomerate transformation lacks precedent in sublimed amino acids, it is not complete oddity and has been apparently observed in a few cases.[17,18] ¶

Herein we extend this asymmetric amplification to other amino acids and their mixtures. Fig. 1 shows the amino acids assessed through this study: alanine, isoleucine, leucine and valine. Like valine, isoleucine (a racemic compound) converted after sublimation into a conglomerate phase, while leucine and alanine (both crystallizing as racemic compounds as well) did not undergo that transformation. This implies that resolution of these amino acids cannot be achieved by sublimation. Conversely, a scalemic sample of isoleucine with an initial enantiomeric imbalance underwent further enantioenrichment.‖ Distinctive features of

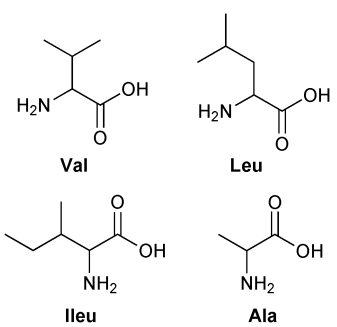

Fig. 1 Amino acid skeletons evaluated in sublimation experiments.

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Table 1 Enantioenrichment of amino acid mixtures by sublimation

Scalemic samples[a] (% ee)	Sublimed material[b] (% ee)
Val-Leu (40%) (L)	Val (54) (L)/Leu (51) (L)
Val-Leu (40%) (D)	Val (52) (D)/Leu (50) (D)
Ileu-Leu (40%) (L)	Ileu (54) (L)/Leu (64) (L)
Val-Ileu (40%) (L)	Val (57) (L)/Ileu (57) (L)
Val-Ileu (57%) (L)	Val (68) (L)/Ileu (69) (L)
Ileu-Ala (40%) (L)	Ileu (55) (L)/Ala (44) (L)

[a] Prepared from LD (racemic compound) and the L-enantiomer (excess) for every amino acid, unless otherwise specified (entry 2). [b] Determined by chiral HPLC; average enantioenrichment of the amino acid constituents in the sublimate mass.

this sublimation have been outlined,[16] and imply that the solid undergoes a sudden sublimation leading to a dense cloud of condensing crystals for a few minutes, which are deposited on the walls (complete transformation to a conglomerate occurs). Once deposited, isoleucine crystals underwent a slow sublimation moving upwards and without any further condensation from the gas phase; *i.e.* the sublimed material deposited on pre-existing crystals. Enrichment took place at this stage. As expected, the highest ee was observed at the front sublimation line and decreased gradually on the top of the wall.

We wondered whether this amplification would be possible in mixtures of two amino acids, even if one of them were unable to attain the conglomerate state. Results collected in Table 1 reveal that this conjecture is indeed the case and modest, yet significant, enrichments occur in both amino acids after one sublimation cycle run in closed vessels (see ESI‡).

Ees were determined by HPLC on a chiral stationary phase and represent average values of each amino acid in the entire sublimate. Thus, a mixture of valine and leucine (40% ee each, L-enriched samples) gave rise to a sublimed material containing the same amino acids in 54% and 51% ee, respectively. Similar enrichments could be observed in a scalemic sample containing the D-enantiomer in excess (entry 2). Leucine, which did not exhibit any enrichment alone, experienced a further improvement (64% ee) when sublimed in combination with isoleucine. Alanine (the simplest chiral amino acid) was quite reluctant to undergo this sort of resolution, either with valine or isoleucine, and a poor increase in ee (*ca.* 4% on average) could be measured in the presence of scalemic isoleucine (Table 1, last entry).

Moreover, one could figure out that in a natural scenario, recycling would enhance the enantioenrichment still further. This surmise works well with valine that undergoes spontaneous resolution *via* conglomerate formation. Starting again from an L-enriched scalemic sample of 40% ee, the sublimate gave rise to an average enrichment of 56% ee. The crystalline mass was cooled to room temperature and dissolved in water. Evaporation of the latter and subsequent sublimation at the same temperature yielded a material with 69% ee, which could further be improved up to 80% ee after the third cycle. Gratifyingly, this trend was corroborated when an enriched mixture of valine plus isoleucine was subjected to two consecutive sublimation cycles leading to approximately the same enantioenrichment (1st: 57% ee; 2nd: 68% ee) of both amino acids (Table 1, entries 4 and 5).

At this stage the mechanism responsible for the enantioenrichment of amino acids under the above-mentioned protocol is unclear, but it seems to be unrelated to a fractional disproportionation where either the racemate or the pure enantiomer sublimes preferentially due to vapor pressure differences between stereoisomers. Rather, it is plausible to anticipate any kind of enantiomerization which might take place on the wall or the gas–solid interface. Our observations also indicate that an L- or D-rich conglomerate amino acid apparently induces further enantioselection in another amino acid forming heterochiral crystals. The specific homo- and

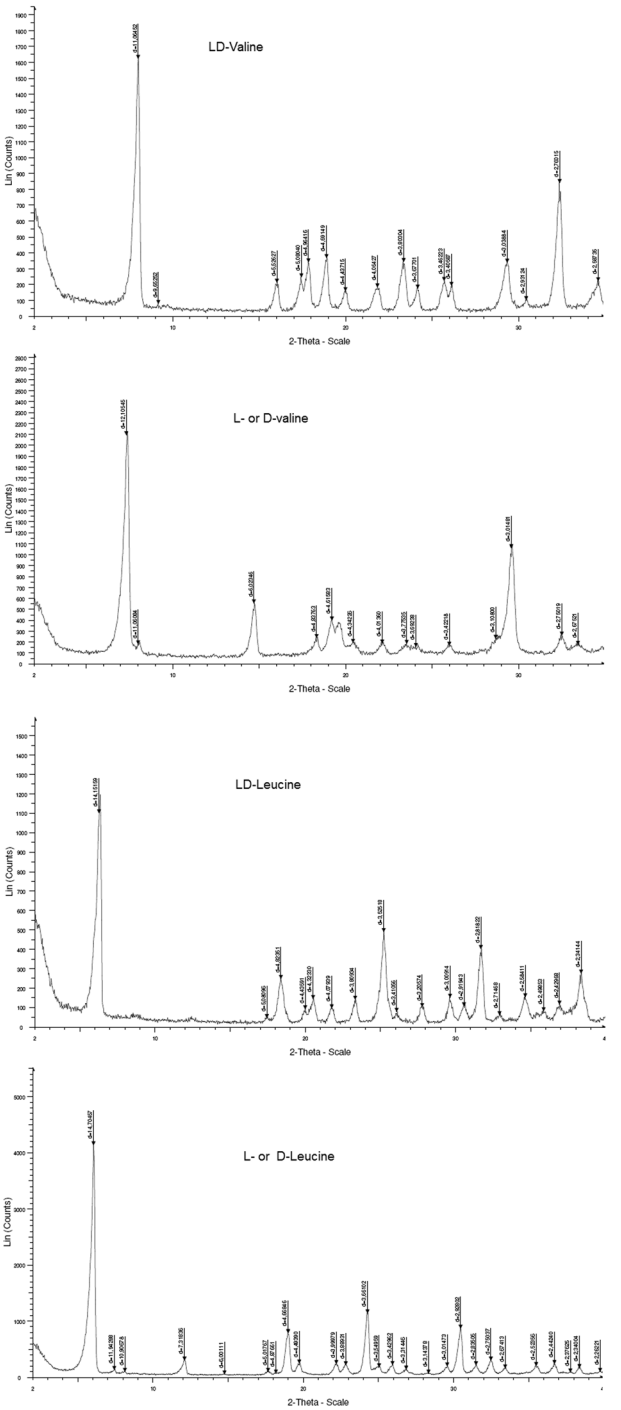

Fig. 2 Top: powder X-ray diffractograms of LD-valine before sublimation (racemic compound) and that of homochiral valine; bottom: powder XRD of LD-leucine prior to sublimation (a racemic compound as well) and that of pure enantiomers.

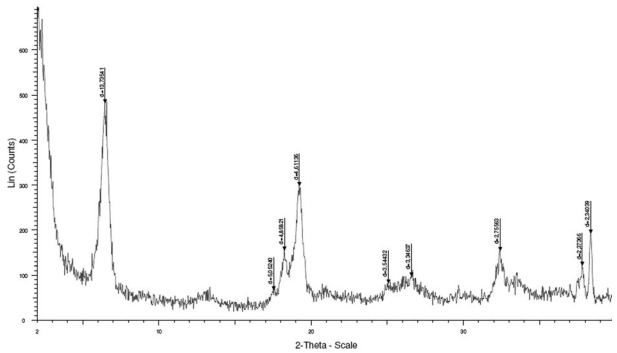

Fig. 3 PXR-diffractogram of Leu-Val after sublimation.

hetero-chiral interactions of such species in the gas-phase wait for future hindsights. Powder X-ray (PXR) diffraction was again instrumental in assessing unequivocally the racemic compound to conglomerate switch. As depicted in Fig. 2 and 3 the two amino acid-sublimate of valine–leucine shows peaks at 2θ and *d*-spacing values that are markedly different from those of both the racemate and the pure enantiomer from either amino acid. This observation would point to further structural changes. In the case of valine–alanine, the diffractogram after sublimation did not show any peak attributable to the parent alanine.[19]**

Clearly, there is still a long way to understand the extreme Nature's unichirality. We have conclusively shown that sublimation of amino acids possessing a geochemically credible enantiomeric imbalance leads to an enhanced enantioenrichment of the entire mass. Further improvements having a look at environmental conditions are under way.

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Notes and references

§ Unichiral denotes unambiguously an enantiomerically pure substance and should therefore be the term of choice when this connotation is invoked; see an illuminating paper for further discussion: J. Gal, *Enantiomer*, 1998, **3**, 263–273. Homochiral is not synonymous to enantiomerically pure, although there is no ambiguity in the present paper and we use it as such quoting the original sources.

¶ Ref. 17 is most likely the first example reporting spontaneous resolution after conglomerate formation. Thus, sublimation of a racemic terpenol (a non-crystallizable solid) gave rise to crystal clusters, some of which were dextrorotatory and other levorotatory. This clearly requires that such *rac*-alcohol is a conglomerate whose enantiomers nucleate independently on the wall of the vessel. However, as highlighted by Eliel and Wilen: "while the racemate should have exhibited this spontaneous resolution, just as well prior to sublimation, the absence of crystals in the crude product prevented establishment of the fact that the product was [already] a conglomerate"; see E. L. Eliel and S. H. Wilen, *Stereochemistry of Organic Compounds*, John Wiley & Sons, New York, 1994, p. 299.

‖ All sublimation experiments have been carried out in glass vessels heated in a conductive plate. The measured temperature at the bottom in contact with the heater was *ca.* 430 °C (see ref. 16). Although the process can be conducted at lower (external) temperatures; the latter causes the appropriate thermal gradient and the whole solid to sublime almost instantly and condense on the upper and colder zone of the vessel.

** It is known that L-alanine is capable of forming a co-crystal with the small dipeptide L-Ala-L-Val. The co-crystal has a layered structure and alanine molecules occupy the voids of a β-sheet structure. See ref. 19.

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